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Fine Structural and Morphometric Studies of the Merkel Cell During Fetal and Postnatal Development

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The morphological and morphometric changes of the Merkel cells during fetal and postnatal development were studied in the glabrous digital pads of rats. In 20-day-old fetus rats, the Merkel cells we observed were present in the lower spinous and basal layers, and not associated with axon terminals. The Merkel cell granules were few and sparse. The Merkel cell had clumps of fibrils and formed many desmosomes with surrounding keratinocytes. In postnatal rats, innervation was followed by an increase in the number of Merkel cell granules, and their specific accumulation. The fibrils of the Merkel cells were not prominent. It was found by *t*-test that the numerical density of the Merkel cell granules significantly increased from the fetal stage to 4-day-old postnatal rats. These results suggest that the Merkel cells are present in the epidermis without nerve contact in 20-day-old fetus rats, and that innervation is necessary for the increase of cellular activity in Merkel cells.

INTRODUCTION

Merkel cells are thought to be special tactile cells [1] in the epidermis, and their fine structure has been well documented by electron microscopic studies on various mammals [2–11]. In mature mammals [12–16], the typical Merkel cells are situated in the basal layers, and attached to meniscoid axon terminals. They contain Merkel cell granules in the cytoplasm which accumulate in the direction of the nerve attachment, and form many desmosomes with surrounding keratinocytes.

However, we still know very little about the morphological and morphometric changes of Merkel cells during their development from the fetal to adult stage.

The present study was undertaken to determine the nature of the electron microscopic changes in the Merkel cells of rats during their development.

MATERIALS AND METHODS

A total of 30 animals were used in this study, 5 animals for each of 6 developmental stages (20-day-old fetus, 2, 4, 7, and 14-day postnatal and adult rat). Skin samples were taken from the glabrous digital pads of the rats, fixed with 2.5% glutaraldehyde and 2% osmium tetroxide buffered with phosphate (pH 7.4) for 2 hr, dehydrated in ethanol, and embedded in a mixture of Epon and Araldite. Thin sections were cut with an LKB 8800 Ultrotome III, stained with lead citrate and uranyl acetate, and examined with a JEM 100B electron microscope.

Morphometry was performed on electron micrographs enlarged to a magnification of 15,000 to 20,000 times. A SEIKO 5900 Personal Computer (32KB) and NAC Graf/pen along with a BCD interface were used for planimetry and counting the number of granules.

Statistical analyses were carried out by *t*-test.

RESULTS

We found a sum total of 15 Merkel cells in the 20-day-old fetuses, 14 cells in the 2-day, 20 cells in the 4-day, 14 cells in the 7-day, 10 cells in the 14 day, and 14 cells in the adult rats.

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Fine Structure of the Merkel Cells

20-day-old fetus rats: The Merkel cells are present in the basal layer and the lower portion of the spinous layer in the epidermis, and are the same size or slightly larger than the associated keratinocytes (Fig. 1*A*). They have slightly convoluted nuclei. The cell boundary is oval or round, and the cytoplasm is similar in electron density to that of the adjacent keratinocytes. The rough-surfaced endoplasmic reticulum is poorly developed, but free ribosomes are numerous. In some Merkel cells, glycogen particles are observed. Golgi apparatuses are present, but not prominent (Fig 1*B*). Multivesicular bodies and large dense lysosomal-like bodies are found. The Merkel cells have clumps of fibrils in their cytoplasm in just the same way as keratinocytes (Fig 1*B*). They contain membrane-enclosed dense-core granules (Merkel cell granules), which are 118.0 ± 34.3 nm in diameter. Merkel cell granules in the cytoplasm are few and sparse. Occasionally, the cilium bulges from the Merkel cell into the keratinocyte (Fig. 1*B*). The shaft of the cilium is about 260 nm in width. Longitudinal striations are observed within the shaft. The Merkel cells are attached to adjacent keratinocytes by desmosomes, which are finer and less numerous than those formed between keratinocytes. Axons, where found, are surrounded by keratinocytes, but are not in direct contact with the Merkel cells.

2-day-old postnatal rats: At this stage, the Merkel cells have relatively smooth cell boundaries and they are situated in the basal layer or lower spinous layer (Fig 2). The nucleus is generally round in shape. The cytoplasm contains mitochondria, centrioles, large dense lysosomal-like bodies and bundles of clumped fibrils. In 2-day-old postnatal rats, 2 Merkel cells out of 14 were associated with axon terminals (14%). Occasionally axon terminals are surrounded by the cytoplasmic projections of the Merkel cells (Fig 3). The granules, which are 67.3 ± 19.4 nm in diameter, are few and sparse in the nerve-associated Merkel cells, and very similar to those in the non-associated cells. Between the Merkel cell and adjacent keratinocytes, poorly developed desmosomes are observed (Fig 3).

4, 7, and 14-day-old postnatal rats: As in 2-day-old rats, the Merkel cells are situated in the basal layer or lower epidermis. In these stages, the Merkel cells begin to show some difference in fine structure and it becomes possible to make a distinction between 2 main types (Fig 4). Cells of the first type are similar to those in the 20-day-old fetus and 2-day-old postnatal rats. They are oval shaped and have nonconvoluted nuclei. No specific accumulation of the Merkel cell granules is observed. Axons are not associated with them. The bundles of clumped fibrils are well developed (Fig 5). The fibrils in the Merkel cells are 4.0 ± 1.1 nm in width, whereas the tonofilaments in neighboring keratinocytes are 4.2 ± 1.0 nm. When the width of the fibrils was compared with that of the tonofilaments by *t*-test, the fibrils were found to be the same width as the tonofilaments ($p < 0.05$). The cells of the second type have smooth cell boundaries and lobulated nuclei (Fig 4). The fibrils in the cytoplasm are not as prominent as those in the former cells. Most of the Merkel cells are associated with axon terminals. They contain numerous granules which polarize towards the axons. The Golgi apparatus is opposite the side where the granules accumulate. Both cells form numerous desmosomes with the surrounding keratinocytes.

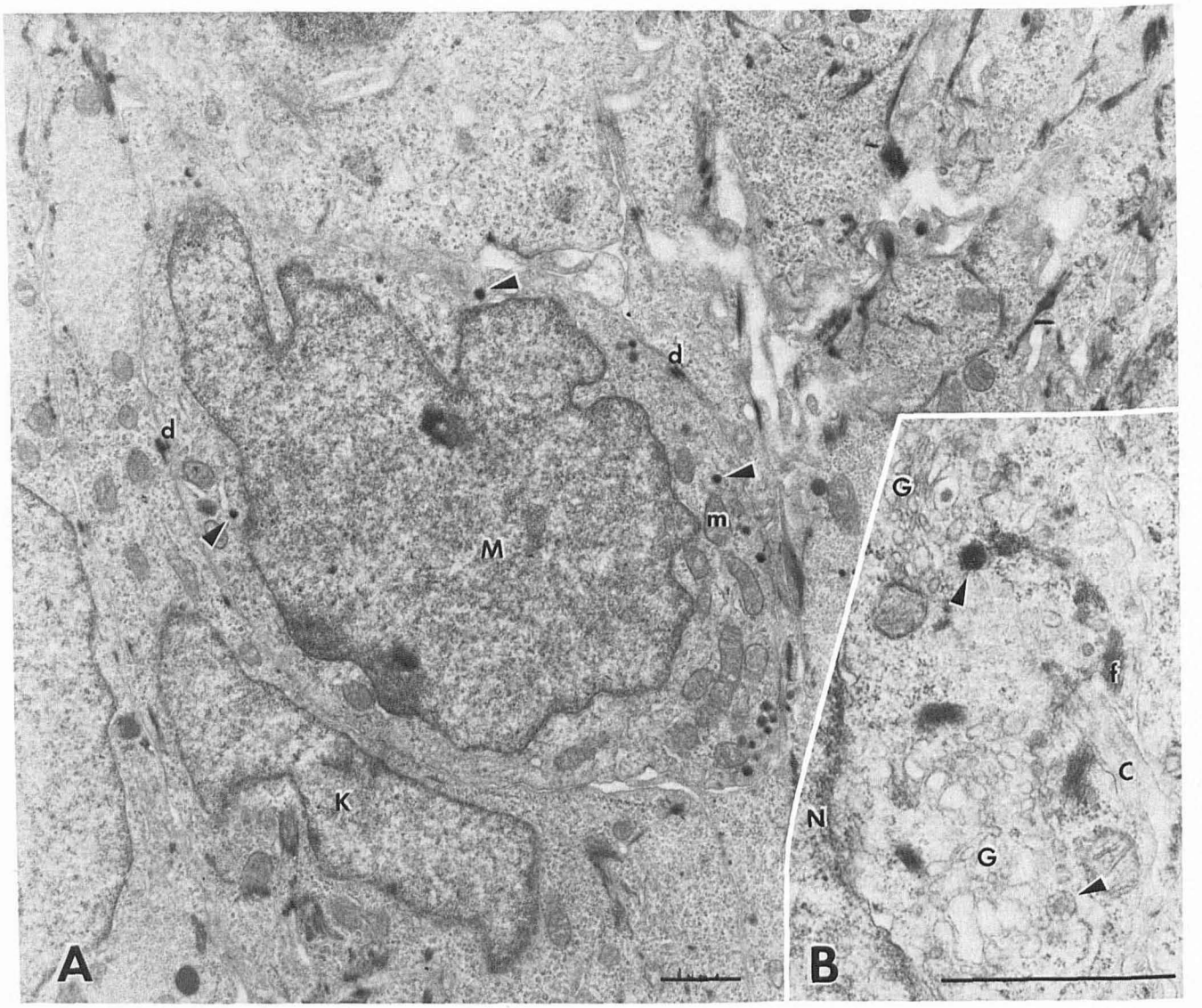

FIG 1. Merkel cells (*M*) in the lower epidermis of a 20-day-old fetus rat. *A*, The Merkel cell granules (*arrow heads*) are few and sparse. Axons are not associated with it. *m*, mitochondria; *K*, keratinocyte, *d*, desmosome (× 10,600). *Scale bar*: 1 μm. *B*, Cilium (*C*) and clumps of fibrils (*f*) are found in the cytoplasm. *G*, Golgi apparatus. *N*, nucleus (× 33,800). *Scale bar*: 1 μm.

Adult rats. At this stage, the Merkel cells are found in the basal layer distributed among the keratinocytes to form an alternating pattern of Merkel cells and keratinocytes (Fig 6). The cytoplasm is elongated, and the nucleus is highly lobulated. They are oriented with the long axis perpendicular to the basal lamina. The cytoplasm contains irregularly shaped mitochondria, large dense lysosomal-like bodies and Golgi apparatuses. The fibrils are few and scattered. The Merkel cell granules, about 64.0 ± 17.9 nm in diameter, are mainly concentrated on the side of the cytoplasm near the axon terminals, and well away from the Golgi apparatus. In adult rat fingers, all the Merkel cells we observed are associated with meniscoid axons. The Merkel cells are attached to adjacent keratinocytes by desmosomes, and to the basal lamina by half desmosomes (Fig 7).

Changes in Numerical Density of the Merkel Cell Granules with Aging (Fig 8)

At each stage, the numerical density of the granules, i.e., the number of granules divided by the area of cytoplasm was computed morphometrically. Numerical density was 0.42 (number of granules/μm^2) in the 20-day-old fetus rats, and increased from fetus to 4-day-old postnatal rats. However, when the numerical density in the 4-day-old rats (2.45/μm^2) was compared with that in older rats by *t*-test, the Merkel cell granules were not found to show any significant increase in density with aging after the 4th day ($p < 0.05$).

Changes in the Diameter of the Merkel Cell Granules with Aging (Fig 9, Table I)

The diameter of the core of all the Merkel cell granules was measured at each stage. The values are shown in both the Table and Fig 9. When the diameter of the Merkel cell granules in the 20-day-old rat (118.0 ± 34.3 nm) was compared with that in the postnatal rats by *t*-test, it was found that the diameter was significantly smaller after birth ($p < 0.05$).

DISCUSSION

We examined the various changes that take place in the Merkel cells of rats at various stages from the fetus to the adult. Although Merkel cells are generally thought of as nerve-associating cells, the presence of nonassociating Merkel cells has been previously reported. English [17] observed the rat Haarscheiben, and demonstrated the presence of Merkel cells with-

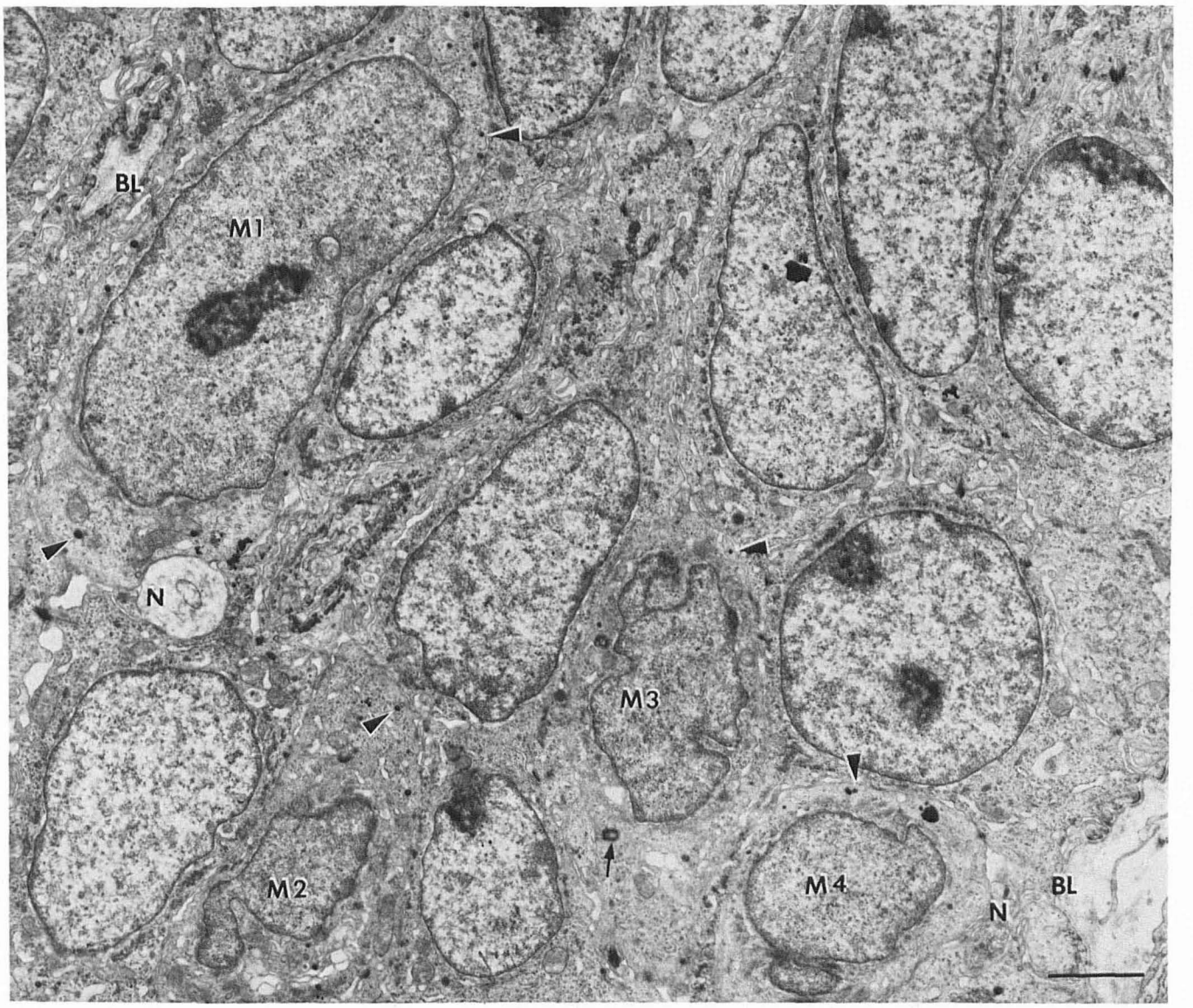

FIG 2. Four Merkel cells (*M1, M2, M3, M4*) in a 2-day-old postnatal rat. They have relatively smooth cell boundaries. A few Merkel cell granules (*arrow heads*) are seen. Two Merkel cells (*M1, M4*) are associated with axon terminals (*N*). *Arrow*, centriole, *BL*, basal lamina (× 6,500). *Scale bar*: 2 μm.

out visible nerve contacts in newborn and 13-day-old rat Haarscheiben. Lyne and Hollis [18] observed sheep fetuses ranging in age from 33 to 144 days gestation, and showed isolated Merkel cells without axon terminals in the epidermis of 57-day-old fetuses, in which the Haarscheiben was not found. Breathnach [19] reported that some Merkel cells in the human fetus (16 weeks) were not associated with axon terminals. We were able to observe in the present study the presence of Merkel cells which were not associated with axons in the glabrous digital pads of 20 day fetuses and postnatal rats. This shows that in the case of rats Merkel cells can exist without contact with axon terminals. These nonassociated Merkel cells showed the following electron microscopic characteristics: (1) They were located in the lower spinous and basal layers, and had, (2) a lightly staining nucleus and cytoplasm, (3) nonconvoluted nucleus, (4) a few scattered Merkel cell granules without specific accumulation, and (5) clumps of fibrils similar to the tonofilaments of keratinocytes both morphologically and morphometrically. In our investigation, nerve-associated Merkel cells were first observed in the 2 day postnatal specimens, which suggests that in the case of rats the Merkel cells begin to associate with the axons at some point after the 20th day of gestation. However, serial sections would be necessary in order to be certain of this.

In the 20-day-old fetus, when the Merkel cells are still not yet attached to the axon terminals, the Merkel cell granules were few in number and scattered throughout the cytoplasm. During the period from fetus to 4 days after birth it was verified by *t*-test that there was a significant increase in the number of Merkel cell granules, as axons become attached to the Merkel cells. After the 4 day postnatal stage, however, there was no further significant change in the number of Merkel cell granules. These results show that by the 4 days postnatal stage there is a significant increase in the number of Merkel cell granules, and there seems to be an accumulation of these granules in the direction of the nerve attachment. This would strongly suggest that innervation is necessary for the increase of cellular activity in the Merkel cells. Further evidence to support this view can be found from other sources. English [20,21] showed that cutaneous nerve transection is followed by a degeneration of the Merkel cells and atrophy of the Haarscheiben supplied by the nerve in adult cats. She further reported that sensory nerves

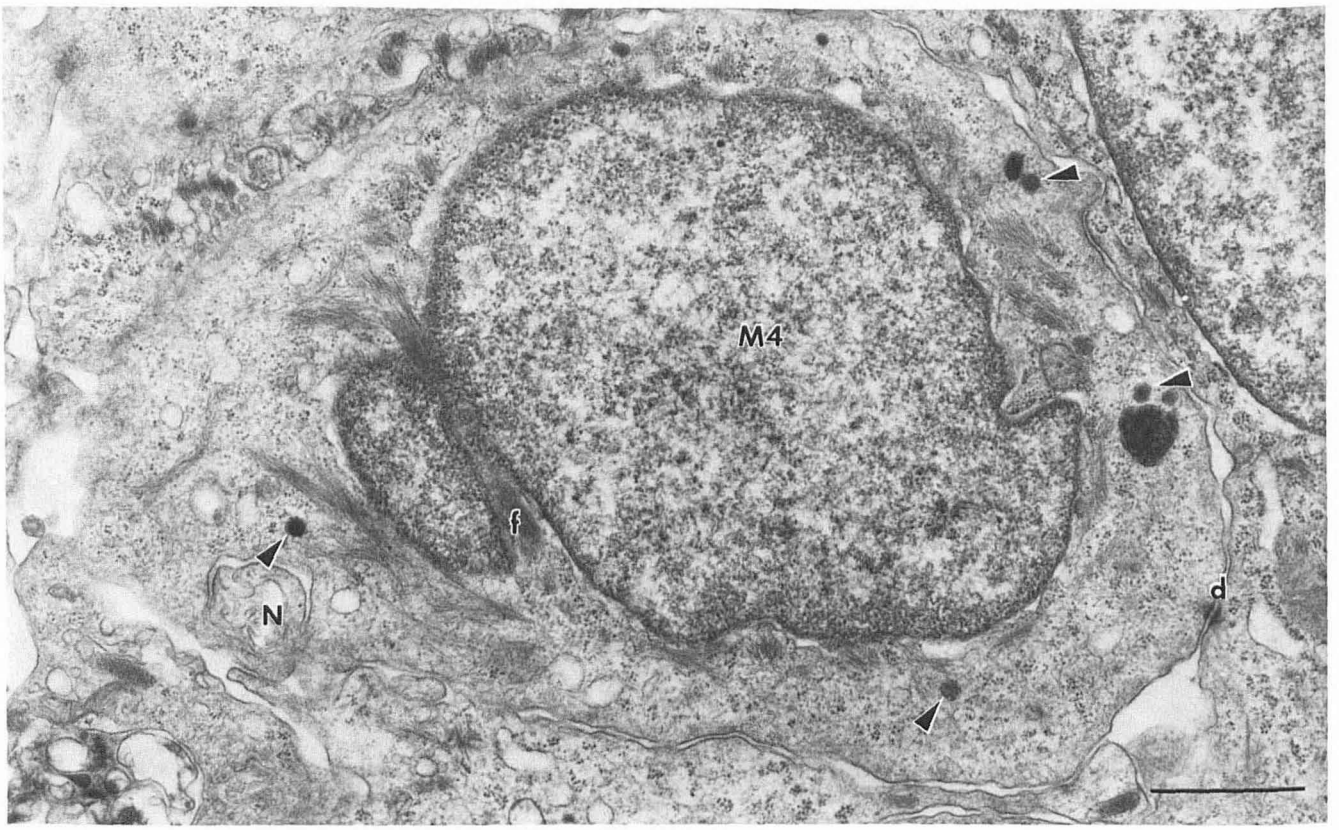

FIG 3. Merkel cell (*M4*) in a 2-day-old rat. A few scattered Merkel cell granules (*arrow head*) and clumps of fibrils (*f*) are seen. The axon terminal (*N*) is surrounded by cytoplasmic projection of the Merkel cell. *d*, desmosome (× 21,000). *Scale bar*: 1 μm.

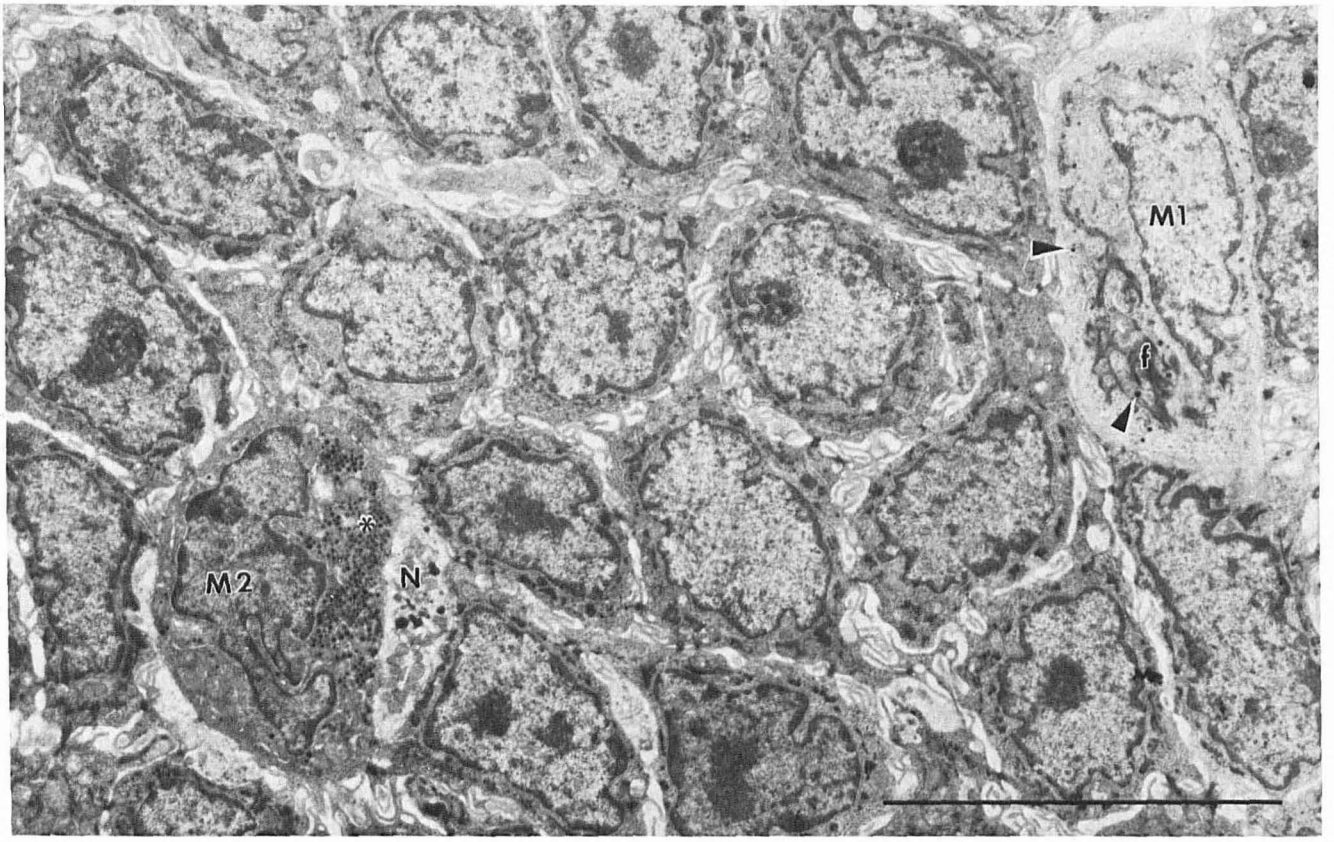

FIG 4. Two types of Merkel cells (*M1*, *M2*) in a 7-day-old rat. In *M1*, Merkel cell granules (*arrow head*) are sparse and few. The bundles of fibrils (*f*) are well developed. Axons are not associated with *M1*. In contrast, in *M2*, a specific accumulation of the granules (*) is found in the region of the meniscoid nerve attachment. The fibrils are not prominent. *N*, axon terminal (× 5,200). *Scale bar*: 10 μm.

"trophically" influence the structure of the Merkel cells and Haarscheiben receptors. After nerve transection, the Merkel cell granules decreased in number, in the cat [21], opossum [22], and mouse [23], and it was also reported that the specific accumulation of the granules was lost [24]. Kurosumi et al. [24], however, suggested that the maintenance of the Merkel cell does not depend entirely on the presence of nerve fibers, because the Merkel cell granules never disappeared even after a long period of denervation.

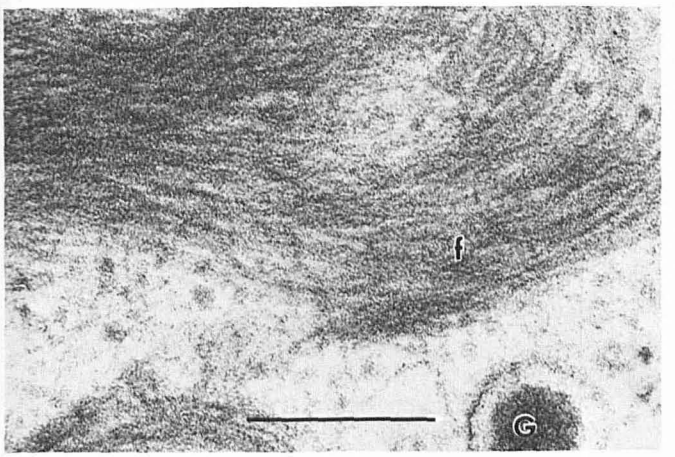

FIG 5. Clumps of fibrils (*f*) in the Merkel cell of a 7-day-old rat. The fibrils are 4.0 ± 1.1 nm in width. The tonofilaments in the surrounding keratinocytes are 4.2 ± 1.0 nm. *G*, Merkel cell granule (× 120,000). *Scale bar*: 0.2 μm.

The origin of the Merkel cell is still unknown. Munger [25], and Kurosumi et al [26] considered the Merkel cells to be modified epidermal cells, since they were joined to keratinocytes with desmosomes. Lyne and Hollis [18] also stated that Merkel cells originate from epidermal cells, because the isolated Merkel cells in sheep fetus were attached to keratinocytes by desmosomes. English [17] supported the view that Merkel cells may be keratinocytic because transitional cells were conspicuous in the developing Haarscheiben of rats. The results of the present investigation also seem to support the view that Merkel cells may originate from keratinocytes. First, in the 20-day-old fetus Merkel cells were found only in the epidermis, and they were all nonnerve associated Merkel cells. We found many such "transitional" cells [17] in the fetus and postnatal stages, and both transitional and typical Merkel cells at later stages. No dermal Merkel cells were found. These seem to suggest strongly that the Merkel cells originate from keratinocytes. Furthermore, Merkel cells were found to attach themselves to keratinocytes and basal lamina by desmosomes and half desmosomes respectively, in exactly the same way as ordinary keratinocytes. This similarity in the manner of their associating may perhaps be used to support the theory that they are essentially of the same origin. Although we cannot expect to fully understand and establish the nature of the origin of the Merkel cell by morphological evidence alone, the results of this study seem to suggest that Merkel cells originate from epithelial cells such as keratinocytes in epidermis.

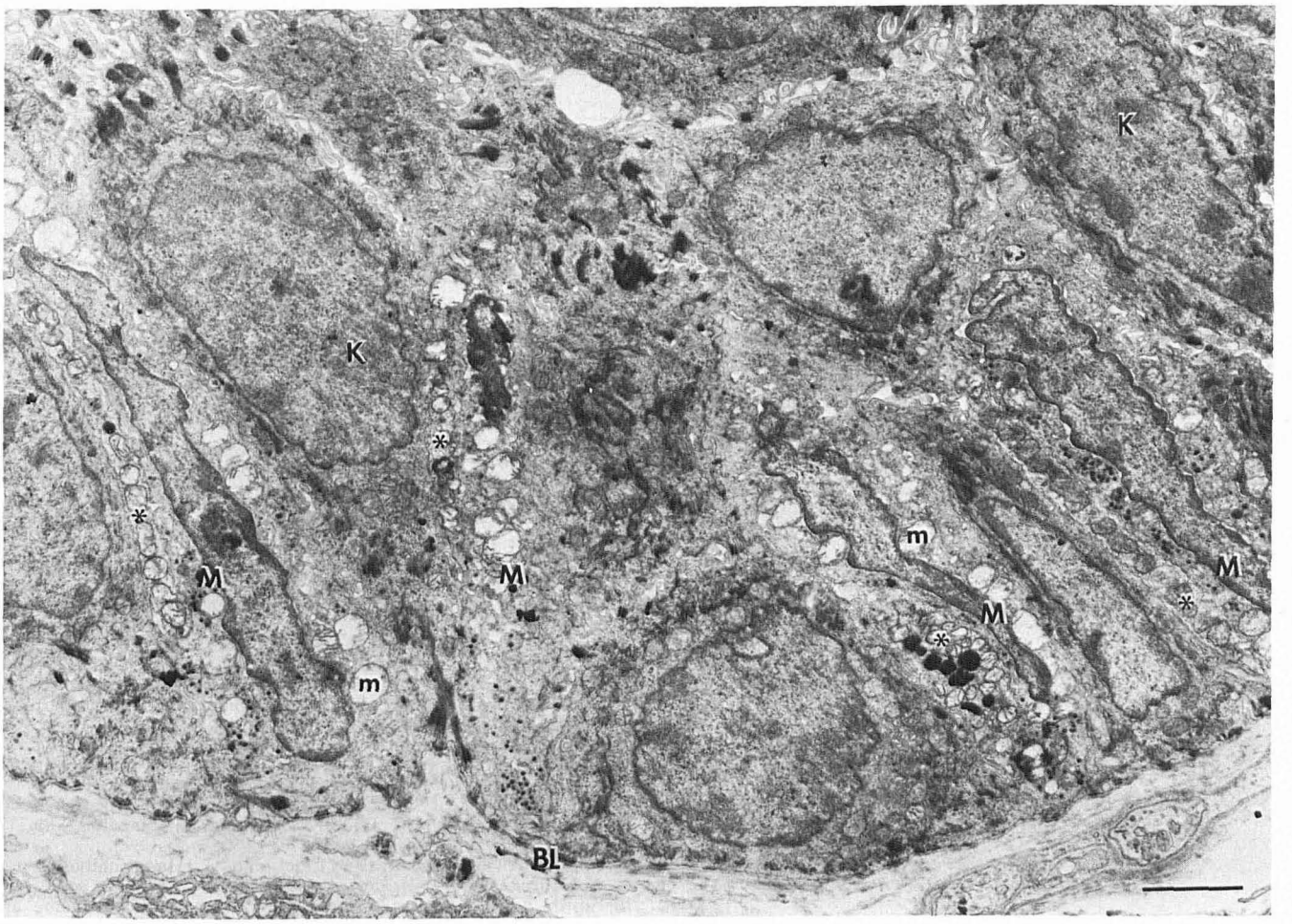

FIG 6. Four Merkel cells (*M*) in an adult rat. The Merkel cells are present in the basal layer and have elongated nuclei and cytoplasms. All of them are associated with axon terminals (*). *m*, mitochondria. *BL*, basal lamina. *K*, keratinocyte (× 6,500). *Scale bar*: 2 μm.

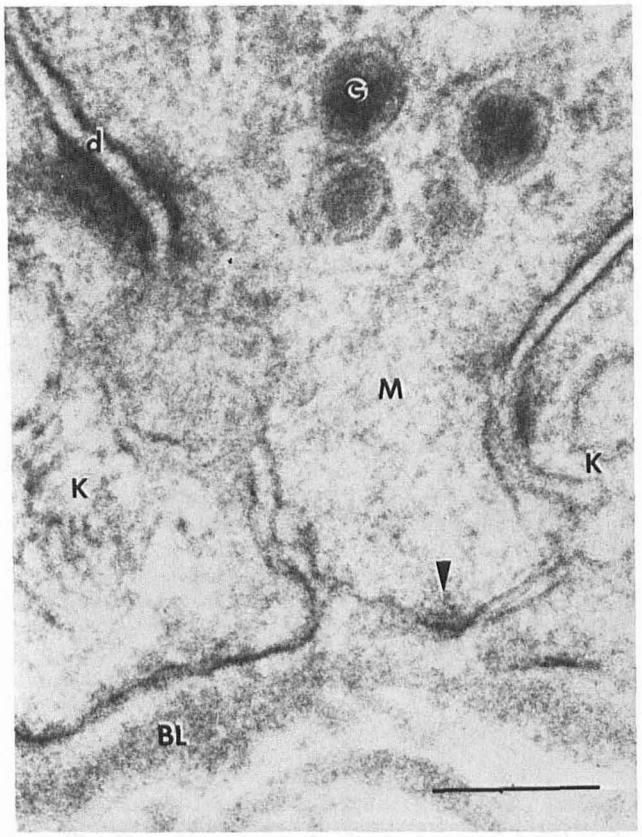

FIG 7. The Merkel cell (*M*) in an adult rat. The Merkel cell is attached to adjacent keratinocyte (*K*) by desmosomes (*d*), and to the basal lamina (*BL*) by half desmosomes (*arrow head*). *G*, Merkel cell granules (× 112,000). *Scale bar*: 0.2 μm.

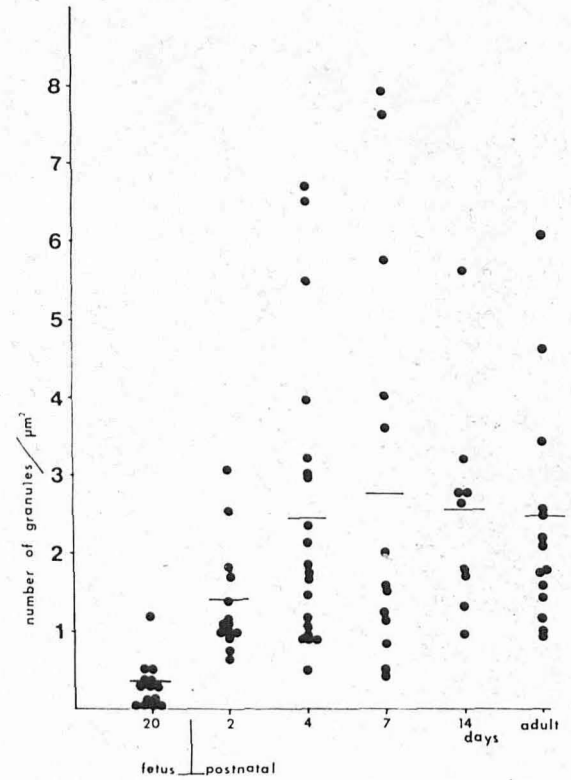

FIG 8. Numerical density of the Merkel cell granules during their fetal and postnatal development. The granules increase in number from the 20-day-old fetus to 4-day-old postnatal stage. ——: mean value.

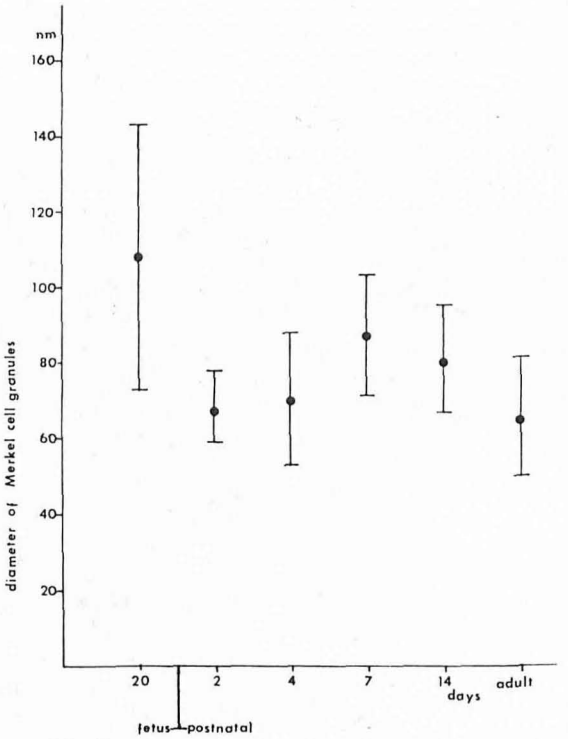

FIG 9. The diameter of the Merkel cell granules in rats during fetal and postnatal development. There is reduction in the diameter of the granules after birth.

Diameter of the Merkel cell granules from the fetal to adult stage

	Mean ± SD (nm)	Minimum (nm)	Maximum (nm)
20-day-old fetus rat	118.0 ± 34.3	41.1	207.0
2-day-old postnatal rat	67.3 ± 19.4	30.4	123.0
4-day-old postnatal rat	70.8 ± 16.2	30.5	151.3
7-day-old postnatal rat	86.4 ± 16.8	30.5	138.7
14-day-old postnatal rat	79.1 ± 14.7	34.7	156.1
Adult rat	64.0 ± 17.9	31.9	139.0

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Announcement

The Japanese Society for Investigative Dermatology will hold an International Workshop of Investigative Dermatology in Kyoto, Japan, May 31–June 1, 1982, as a "Post-Congressional Satellite Meeting" to the XVI International Congress of Dermatology. The topics will include photobiology, melanogenesis, immunology, hormones, inflammation, keratinization, virology and bacteriology, electron microscopy and membrane receptors. Applications to attend the Workshop must be received before January 31, 1982. The language for the Workshop will be English.

A travel fellowship of U.S. $200 per person for this "Satellite Meeting" is being made available for qualifying active foreign participants. Please apply, with an 800 word abstract for your presentation, and curriculum vitae including a bibliography of the last 3 years, to arrive not later than January 31, 1982.

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